STEROID PATHWAY GENES AND NEONATAL RESPIRATORY DISTRESS AFTER BETAMETHASONE USE IN ANTICIPATED PRETERM BIRTH

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Author Contributions

DH and DF designed the original research; DH performed the research; DH, SS, KT, TF designed the current analysis; DH, JT, DL, SS, AK, KT, and TF performed analyses and data interpretation. All authors contributed to writing the manuscript and approve of this final version.

Abstract

Objective: To test several key glucocorticoid genes that are enhanced in lung development for associations with respiratory distress syndrome (RDS) after antenatal corticosteroid use.

Methods: A prospective cohort of women received betamethasone to accelerate fetal lung maturity for threatened preterm delivery. DNA was obtained from mothers and newborns. Neonatal RDS was the primary outcome. Genotyping for SNPs in 68 glucocorticoid genes found to be differentially expressed during lung development was performed. Multivariable analysis tested for associations of SNPs in the candidate genes with RDS.

Results: Genotypic results for 867 SNPs in 96 mothers and 73 babies were included. 39 babies (53.4%) developed RDS. Maternal SNPs in the *CENPE, GLRX, CD9*, and *AURKA* genes provided evidence of association with RDS ($p<0.01$). In newborns, SNPs in *COL4A3, BHLHE40*, and *SRGN* provided evidence of association with RDS ($p<0.01$).

Conclusion: SNPs in several glucocorticoid responsive genes suggest association with neonatal RDS after antenatal corticosteroid use.

Key words- antenatal corticosteroid, pharmacogenetics, pregnancy, preterm labor

Introduction

The benefits of antenatal corticosteroids for the preterm infant are clear. The reduction in mortality and morbidity has been a major advance in reducing the cost of preterm birth.[1] As with many therapeutics, however, not all babies receive the same benefit from antenatal corticosteroids.[2] We have previously demonstrated that genetic polymorphisms in key drug metabolism and steroid receptor pathways can account for some of the differences in neonatal respiratory outcomes.[3,4]

The pathways of steroid response are complex. While our prior studies demonstrated that single nucleotide polymorphisms (SNPs) in *adenylate cyclase 9* (*ADCY9*) and *Importin 13* (*IPO13*) are associated with respiratory distress syndrome (RDS),[3,4] reports from other complex respiratory diseases such as asthma, have uncovered multiple genes involved in respiratory response to steroids.[5,6] A group of steroid genes have been demonstrated to be up-regulated and important in human fetal lung development.[7] The importance of these steroid pathway genes in disease development, however, is not yet known. A larger scale investigation of the role of steroid pathway genes in the development of RDS in a population receiving antenatal corticosteroids has not been performed according to our knowledge.

The objective of this study was to test for associations of specific key glucocorticoid genes that are differentially expressed during lung development with neonatal RDS after antenatal corticosteroid use for anticipated preterm birth. The hypothesis was that key steroid genes in either the mother or developing baby would impact the effectiveness of antenatal corticosteroids that thus would be associated with development of RDS. Finding key steroid genes associated with RDS may then enable better understanding of how antenatal corticosteroids enhance lung maturation in an effort to further improve this therapy.

Methods

Subjects and Samples

This was a secondary analysis of a betamethasone (BMZ) pharmacogenetics cohort study. The acquisition of the cohort and samples has been documented elsewhere.[4] Briefly, women admitted to the hospital with threatened preterm delivery who received BMZ were recruited to the study. Informed consent was obtained for all women enrolled and the governing Institutional Review Board approved the study. Informed consent was obtained from all participants who were enrolled prospectively as they were admitted to the hospital for BMZ administration. Participants had to be at least 18 years old and at least 23 weeks but less than 34 weeks gestation. Exclusion criteria included known fetal anomaly or inability to provide consent. Standard clinical care at the providers' discretion was provided to the woman and standard neonatal resuscitation and care practices were provided by the pediatric/neonatal services. Women could have received a rescue course of BMZ per routine clinical care if the clinical course warranted. No restrictions were placed on clinical provider care as this was an observational cohort study. The neonatal outcome of RDS was diagnosed by the pediatricians following standard NICHD Neonatal Research Network criteria.[8]

Maternal DNA was obtained from whole blood or from a salivary sample if unable to obtain blood. Neonatal DNA was obtained from umbilical cord blood or from buccal swabs if unable to obtain umbilical cord blood at the time of delivery. Salivary or buccal samples were collected and processed using the Oragene® saliva kit (DNA Genotek). DNA isolation was done according to manufacturer instructions. Samples were frozen at -80˚C until quantified. DNA was extracted from blood samples using the QIAamp® DNA mini kits (Qiagen Inc., Valencia, CA). Manufacturer spin protocol instructions were followed for all kits. When manufacturer protocols listed steps for highly concentrated DNA, those steps were followed. Isolated DNA was transferred into 1.2mL cryovials and all samples were stored at -80˚C until quantified. All quantification was completed using Quant-iT^{TM} Fluorometer and Quant-iT^{TM} dsDNA Broad Range Assay kit (Life Technologies, Inc., Carlsbad, CA)

Glucocorticoid gene selection

We determined a corticosteroid gene set by identifying genes that were differentially expressed between dexamethasone and sham treated immortalized lymphoblastoid cell lines obtained from adolescent subjects with mild-moderate persistent asthma participating in the Childhood Asthma Management Program (CAMP).[9,10] We then identified which of these glucocorticoid genes were enriched in human lung development using human fetal lung tissue samples from the pseudoglandular and canalicular stages of lung development. Briefly, using genome-wide gene expression arrays, generated from 36 human fetal lung tissue samples (post-conception ages 7-26 weeks),[11] we next tested whether the identified steroid response genes were enriched during early human fetal lung development. To do this, principal components analysis (PCA) was performed using the gene expression profiles from human fetal lung tissue. The principal component (PC) that was most highly correlated with gestational age was identified. A chi square test was performed to determine whether the top 5% of genes contributing to the PC most highly associated with gestational age was enriched for genes in the steroid response pathway. In total, 68 genes were differentially expressed at genome-wide significance in CAMP cell lines and were enriched during human lung development and thus were included in the corticosteroid gene set. (Table 1) The selection of these genes was done as part of a different project. The project described in this current study utilized the gene set that was differentially expressed by treatment response in asthmatic subjects and demonstrated enrichment during lung development.[12]

Genotyping

Genotyping for the 68 glucocorticoid response genes previously found to be enhanced in human fetal lung development[7] was performed using the Affymetrix Axiom® Genome-Wide LAT Array (Santa Clara, CA). The Axiom array includes 818,154 probe sets. Non-degraded DNA from the mothers and infants was prepared at concentrations of 15ng/μL and placed into 96-well plates according to the

Affymetrix Axiom® instructions. Degradation of DNA samples was assessed by agarose gel electrophoresis. The genome-wide array was utilized as the most cost-effective method to interrogate the number of genes in the planned analysis. Table 1 lists the genes analyzed. DNA samples from the original cohort that did not have enough volume or could not achieve a high enough concentration for the array were excluded from this study (n=13 women).

Statistical analysis

Given the sample sizes, we only performed analyses for the phenotype of RDS. Analyses were performed testing two hypotheses. The first was that the susceptibility for RDS was associated with maternal genetic variants. The second was that susceptibility for RDS was associated with genetic variants in the baby. For each SNP, we tested whether there was evidence for an association in the mothers and then we also tested for an association in the infant.

In addition to the previously completed sample Quality Control (QC), SNP QC was also performed. SNPs were removed if their genotyping rate fell below 95% completeness and if the Hardy Weinberg Equilibrium p-value was less than 10^{-4}. Because of the small sample size, analyses only included SNPs having a minor allele frequency of at least 10%. Because this was a racially diverse sample, we included two principal components (PC1 and PC2) as covariates to adjust for population stratification in the logistic regression model created. These principal components were derived from the Axiom SNP data by using EIGENSOFT.[13,14] In addition, we also included gestational age as a covariate in the model. This is an exploratory study and so we used a relatively liberal threshold of $p<0.01$ to identify SNPs of interest.

68 genes were the initial focus of the model analysis. SNPs within these genes were the scope of this analysis. A SNP was defined as being 'in the gene' if it is within ±20kb of that gene. This is typically done by our group to ensure that regulatory regions are not missed.[15] Because samples sizes of

both models are very small, SNP QC was calculated separately for each sample. There were a total of 1,903 SNPs in these 68 genes. For analyses of maternal effects and genetic effects in the baby, 820 and 837 SNPs passed QC, respectively (867 SNPs in total). Original array files were transformed in PLINK (V1.07; http://pngu.mgh.harvard.edu/purcell/plink/) format and initial QC was performed. Only samples passing gender (PLINK option – check sex) and relationship (PLINK option – genome to calculate pairwise IBD estimation) checks were included. We then reviewed the population stratification plot anchored with the standard HapMap samples by using PC1 and PC2 calculated from EIGENSOFT[13,14] for ancestry and noted concordance with self-reported race/ethnicity.

Results

The overall characteristics of the cohort are reported elsewhere.[4] Briefly, women in the original cohort had a mean maternal age of 26.5 years-old, had a mean parity of 1.5 and a mean gestational age at receiving the first dose of BMZ of 28.8±3.3 weeks gestation. From the initial cohort of 109 women and 117 neonates, 13 maternal samples were excluded if the DNA failed QC or did not have high enough concentrations or quantity for the GWAS array. A total of 96 mother-infant pairs remained; 32 women had babies diagnosed with RDS and are referred to here as “cases” and 64 women had babies without RDS and are termed “controls”. When testing the hypothesis in the babies, the sample included 39 cases with RDS and 34 controls without RDS. The mean maternal age of this cohort was 25.9±6.2 years. The mean gestational age at receiving BMZ was 29.2±3.1 weeks and the mean gestational age at delivery was 31.8±3.8 weeks. The mean number of days from BMZ initiation to delivery was 24.7±23.4 days. Only 5 women (5.2%) received a rescue dose of BMZ. The ethnic/racial distribution was: Caucasian 46 (47.9%); African American 30 (31.2%); Hispanic 16 (16.7%), Mixed 2 (2.1%), and Indian 1 (1.0%) and Other 1 (1.0%). These data for the subgroup reported here were not different from the overall original cohort. No women had adverse effects of BMZ injections.

Table 2 displays those SNPs that provided evidence of association with neonatal RDS in either the mother or the baby. All association measures are adjusted, controlling for gestational age at delivery. They are not, however, corrected for multiple comparisons. Maternal SNPs in the *CENPE, GLRX, CD9*, and *AURKA* genes provided evidence of association with RDS (Odds Ratio (OR), 95% Confidence Intervals respectively: 0.34 (0.16-0.74); 2.24 (1.24-4.03); 2.74 (1.35-5.56), 3.60 (1.58-8.20)). In the babies, SNPs in *COL4A3, BHLHE40, CENPE*, and *SRGN* provided evidence of association with RDS (OR (95% CI) respectively: 8.07 (1.68-38.76); 4.08 (1.46-11.36); 0.27 (0.09-0.77); 6.17 (1.66-23.01)). Due to sample size issues, we only controlled for gestational age at delivery, the most predictive variable for RDS. We were unable to incorporate other clinical variables such as infant gender into the current model.

Comment

We have shown that glucocorticoid genes that are enriched during the pseudoglandular and canalicular stages of early human lung development harbor polymorphisms that are associated with RDS in newborn infants. . Our results suggest that this set of glucocorticoid genes appear to be important in the pseudoglandular and early canalicular stages of lung development and that abnormal expression of these genes may result in physiologic changes in the structure and/or function of the lung that result in an increase in in post-natal RDS. Furthermore, our data indicate that in women who received antenatal corticosteroid therapy, both the maternal and fetal genotypes in steroid response genes show evidence of association with RDS. The finding of promising genes in both the mother and infant is consistent with other findings.[3,4] These findings may provide evidence for the fetal origin of RDS. These findings complement our prior work in that this study specifically looked at a set of genes known to be enriched in the developing lung and go beyond the glucocorticoid receptor pathways we reported previously.[3,4]

SNPs in these genes may affect how BMZ acts at the level of the developing fetal lung. Many of these steroid responsive genes are in transcriptional pathways. The maternal significant SNPs are involved in maintaining chromosome stability (*CENPE*), and cell cycle regulation and progression (*AURKA*). In addition, *GLXR* is involved in signaling pathways and *CD9* is important in cell adhesion, motility and differentiation. All of the associated SNPs increased the risk of RDS except for *CENPE* (rs6533058) which had a protective effect. The meaning of this finding is unclear given that this gene is associated with maintaining chromosome stability. We were unable to find other reports of clinical or physiological implications for this particular SNP. While these promising SNPs are from different pathways, more work needs to be done to ascertain the mechanistic link with delivery of the premature fetal lung. The fact that these genes were also found to be enhanced in response to dexamethasone in a large asthma data set and are now associated with premature infant respiratory distress strengthens the potential importance of these genes in normal development and function of the human lung and in glucocorticoid response.

Similarly, the fetal SNPs associated with RDS are in seemingly different pathways, with *COL4A3* being a structural component of collagen, *SRGN* regulating hematopoietic cell proteoglycan, *CENP1* maintaining chromosome stability, and *BHLHE40* helping to control cell differentiation. As current mechanisms of fetal lung maturation rely on the differentiation of surfactant-producing pneumocytes, it is possible that *BHLHE40* may be an important gene in this regard. Of note, all four of these genes were identified to be in the top 5% of genes involved in human lung development using PCA and were significantly differentially expressed between the pseudoglandular and canalicular stages of lung development. *COL4A3* expression was significantly higher in the pseudoglandular stage of development when compared to the canalicular stage ($p=0.04$). However, *SRGN* expression was significantly higher in the canalicular period compared to the pseudoglandular stage ($p=0.008$).

Furhermore, *CENPE* also demonstrated significantly higher gene expression in the canalicular period (p=0.01), as did the *BHLHE40* gene (0.009).

We identified that both maternal and infant SNPs in the *Centromeric protein E* gene *(CENPE)* were associated with the development of RDS in the newborn. *CENPE* is located on chromosome 4q24. It encodes a large kinetochore-associated kinesin-like motor protein required for spindle microtubule capture and attachment at the kinetochore during cell division.[17] Previous studies have demonstrated that somatic mutations in this gene result in an increased risk of familial lung cancer.[18] Although little is known about the impact of CENPE in the development of RDS, its role in the maintenance of chromosomal stability may have implications for a variety of chronic respiratory diseases with developmental origins. Additional studies will be needed to identify the precise role of *CENPE* in the development of RDS.

The strength of this work is that it extends findings of respiratory disease (asthma) steroid responsive genes to the area of fetal development genetics and pharmacogenetics. The consistent findings that some of these genes are also associated with neonatal RDS lend credence to their being important in lung development and function. In addition, the odd ratios for these associations are large. Another strength of the study is the rigorous QC that the analysis undertook to ensure that the likelihood of erroneous associations was lessened.

The study is limited by its size. In addition, the use of gene expression profiles from asthmatic subjects in CAMP to identify the initial glucocorticoid gene set may have identified a group of genes that are implicated in asthma pathogenesis and treatment response as opposed to genes involved in RDS. Although we recognize this as a limitation, the use of this cohort would have biased our results towards the null and is less likely to have resulted in false positive results. While we were able to control for gestational age at delivery, we could not control for other variables in the multivariable model. As other

factors like infant gender, ethnicity, and diabetes may also be associated with RDS, in future studies a larger sample size would be needed to adjust for these confounders without the loss of statistical power. In addition, the smaller sample size did not allow us to look at other outcomes including chronic lung disease of the infant, surfactant use, and total respiratory support days. Furthermore, our results are modest and must be replicated in additional samples from a validation cohort. These genes may also be associated with preterm birth and not simply RDS. Other genetic studies looking at SNPs associated with preterm birth have not noted associations with these genes.[19-27] Because we had access to immortalized cell lines in asthmatic populations, we used these data to identify our corticosteroid gene set. However, using differential expression in an asthmatic population is a potential limitation as some corticosteroid genes that are important in RDS may not have been identified in this patient population. Gene expression studies of these SNPs in the cell lines of interest are also needed to further understand these findings.

The study of pharmacogenetics in pregnancy is still in its infancy. Pharmacogenetics in asthma therapy is somewhat more advanced.[5] Utilizing the homology in the complex respiratory disease of asthma with the development of another complex developmental respiratory disease in RDS is appealing. As antenatal corticosteroids are utilized in both conditions- to treat one and prevent the other- pharmacogenetic analogies may become evident. Steroid pathway genes of impact should continue to be studied to help unlock the mechanisms of fetal lung development and the prevention of RDS. Only then can antenatal corticosteroid pharmacogenetics and the hope of more individualized pharmacotherapy move closer to realities.

In conclusion, several maternal and infant genes in steroid responsive pathways appear associated with the development of neonatal RDS. Further development of these pathways and the mechanisms by which they may influence fetal lung development are needed. The use of genetic

analysis tools and informatics can help advance our understanding of therapeutic response in obstetrics. By better understanding fetal lung development and the genetic influence on the pathways of maturation we may realize further advances in optimizing fetal lung development in preterm birth.

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Table 1: List of steroid genes enriched in lung development analyzed in the study

Gene	Name	Function	Locus	EntrezID
KLF9	Kruppel-like factor 9	transcription factor binds to GC box in promoter	9q13	687
TSPO	Translocator protein	mitochondrial protein factor in flow of cholesterol to permit initiation of steroid synthesis	22q13.31	706
CDKN2C	Cyclin-dependent kinase inhibitor 2C	functions as cell growth regulator	1p32	1031
COL4A3	Collagen type IV, alpha 3 (Goodpasture antigen)	structural component collagen	2q36	1285
FCER1G	Fc fragment IgE gamma polypeptide receptor	receptor involved in allergic reactions	1q23	2207
IGFBP4	Insulin-like growth factor binding protein 4	prolongs the half-life of IGFs	17q12	3487
ISG20	Interferon stimulated exonuclease gene	ribosome biogenesis, degrades single-stranded RNA	15q26	3669
MAL	T-cell differentiation protein	role in formation, stabilization, and maintenance of membrane microdomaines	2q13	4118
SRGN	Serglycin	hematopoietic cell granule proteoglycan	10q22.1	5552
S100A6	S100 calcium binding protein A6	cell cycle progression and differentiation	1q21	6277
TUBA4A	Tubulin alpha 4a	microtubule formation	2q35	7277
TUBB4	Tubulin beta 4	microtubule formation	19p13.3	10382
RND1	Rho family GTPase 1	regulates organization of actin cytoskeleton	12q12	27289
BIRC3	Baculoviral IAP repeat containing 3	inhibits apoptosis	11q22	330
BCL3	B-cell CLL/lymphoma 3	proto-oncogene	19q13.1	602
KLF5	Kruppel-like factor 5	transcription factor binds to GC box in promoter	13q22.1	688
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer B-cell inhibitor alpha	involved in inflammatory response	14q13	4792
RGS1	Regulator of G-protein signaling 1	G-protein signaling family	1q31	5996
RRAD	Ras-related associated with diabetes	voltage-regulate Ca channels, inhibits phosphorylation	16q22	6236
TNFAIP3	Tumor necrosis factor	inhibits apoptosis;	6q23	7128

	alpha-induced protein 3	involved in immune and inflammatory response		
RASSF7	Ras associated domain family member 7	regulates microtubule cytoskeleton	11p15.5	8045
BHLHE40	Basic helix-loop-helix family member e40	control of cell differentiation	3p26	8553
IRS2	Insulin receptor substrate 2	mediates effects of insulin and other cytokines	13q34	8660
SPRY1	Sprouty homolog 1	fibroblast growth factor antagonist, may negatively modulate respiratory organogenesis	4q28.1	10252
AGPAT2	1-acylglycerol-3-phosphate O-acytransferase 2	controls 2nd step in phospholipid biosynthesis	9q34.3	10555
UBE2T	Ubiquitin-conjugating enzyme E2T	regulate cell pathways and proteins	1q32.1	29089
C5orf32	Cysteine-rich transmembrane module containing 1	choromosome 5 open reading frame protein	5q31.3	84418
E2F7	E2F transcription factor 7	regulation of cell cycle progression	12q21.2	144455
FKBP5	FK506 binding protein 5	immunoregulation and basic cellular processes	6p21.31	2289
GLRX	Glutaredoxin	antioxidant defense system and signaling pathways	5q14	2745
GUCY1A3	Guanylate cyclase 1 alpha 3	conversion of GTP to GMP, regulation of cell hyperplasia and differentiation	4q31.3	2982
LGALS3	Lectin galactoside-binding soluble 3	apoptosis, cell adhesion, innate immunity	14q22.3	3958
MDK	Midkine	promotes cell growth, angiogenesis	11p11.2	4192
ENC1	Ectodermal-neural cortex 1	oxidative stress response as transcription regulator	5q13	8507
ZEB2	Zinc finger E-box binding homeobox 2	DNA-binding transcriptional repressor	2q22.3	9839
MACF1	Microtubule-actin crosslinking factor 1	cytoskeletal protein; wound healing and epidermal cell migration	1p32	23499
SLC39A6	Solute carrier family 39 member 6	zinc influx transporter	18q12.2	25800
ERRFI1	ERBB receptor feedback inhibitor 1	important in prenatal lung development; modulates response to steroid hormones in	1p36	54206

		uterus		
DDIT4	DNA-damage-inducible transcript 4	inhibits cell growth	10q26.12	54541
KIAA1524	Cancerous inhibitor of PP2A	oncoprotein, promotes cell growth	3q13.13	57650
AHNAK	AHNAK nucleoprotein	neuronal cell differentiation	11q12.2	79026
C7orf23	Chromosome 7 open reading frame 23	chromosome 7 open reading frame protein	7q21.1	79161
ELOVL7	ELOVL fatty acid elongase 7	synthesis of very long chain fatty acids	5q12.1	79993
CCNB1	Cyclin B1	mitosis regulator at the G2/M transition	5q12	891
CD9	CD9 molecule	cell adhesion, motility, and differentiation	12p13.3	928
CEBPD	CCAAT/enhancer binding protein delta	regulation of genes involved in immune and inflammatory response and macrophage activation	8p11	1052
CENPE	Centromere protein E	maintenance of chromosomal stability	4q24	1062
DUSP1	Dual specificity phosphatase 1	cellular response to environmental stress	5q34	1843
HLA-DMA	Major histocompatibility complex class II, DM alpha	acquisition of antigenic peptides	6p21.3	3108
PDK4	Pyruvate dehydrogenase kinase isozyme 4	regulation of glucose metabolism	7q21.3	5166
PLK1	Polo-like kinase 1	regulator of cell cycle progression	16p12.2	5347
SFTPD	Surfactant protein D	lung defense againt inhaled microorganisms	10q22.2	6441
AURKA	Aurora kinase A	cell cycle regulation and progression	20q13	6790
CDC7	Cell division cycle 7 homolog	critical for G1/S transition	1p22	8317
VAMP8	Vessicle-associated membrane protein 8	transport vesicle membranes	2p12	8673
TNFSF10	Tumor necrosis factor ligand superfamily member 10	induces apoptosis	3q26	8743
IER3	Immediate early response 3	protection against apoptosis	6p21.3	8870
CCNB2	Cyclin B2	Cell cycle control at G2/M transition	15q22.2	9133
SPAG5	Sperm associated antigen 5	component of mitotic spindle	17q11.2	10615
TXNIP	Thioredoxin interacting protein	oxidative stress mediator	1q21.1	10628

DKFZP564O0823	Prostate androgen-regulated mucin-like protein	apoptosis resistance	4q13.3-q21.3	25849
DEPDC1	DEP domain containing 1	transcriptional corepressor	1p31.2	55635
CENPK	Centromere protein K	mitotic progression	5p15.2	64105
VASH2	Vasohibin 2	angiogenesis inhibitor	1q32.3	79805
C13orf34	Bora, aurora kinase A activator	activates onset of mitosis	13q22.1	79866
CDCA3	Cell division cycle associated 3	required for entry into mitosis	12p13	83461
SGOL1	Shugoshin-like 1	chromosome adhesion	3p24.3	151648
TUBB2B	Tubulin beta 2B	major component of microtubules	6p25	347733

Names and descriptions functions from GeneCards Human Genome Compendium (www.genecards.org)

Table 2: Summary of steroid genotypes associated with neonatal RDS

SNP	Chr	BP	Gene	Baby Minor Allele Frequency	aOR (95% CI)	Baby p-value	Mother Minor Allele Frequency	aOR (95% CI)	Mother p-value
rs12621551	2	228,118,403	*COL4A3*	0.17	8.07 (1.68-38.76)	**0.0091**	0.21	2.02 (0.86-4.78)	0.1082
rs7610825	3	5,037,635	*BHLHE40(10kb)*	0.49	4.08 (1.46-11.36)	**0.0072**	0.40	1.42 (0.78-2.62)	0.2547
rs6533058	4	104,130,986	*CENPE(11kb)*	0.47	0.27 (0.09-0.77)	**0.0145**	0.41	0.34 (0.16-0.74)	**0.0067**
rs6871090	5	95,133,586	*GLRX(15kb)*	0.37	0.54 (0.21-1.37)	0.1930	0.37	2.24 (1.24-4.03)	**0.0072**
rs17473271	10	70,848,902	*SRGN*	0.19	6.17 (1.66-23.01)	**0.0067**	0.18	1.25 (0.57-2.81)	0.5693
rs735885	12	6,312,663	*CD9*	0.46	1.78 (0.67-4.73)	0.2452	0.37	2.74 (1.35-5.56)	**0.0054**
rs6064387	20	54,968,159	*AURKA*	0.43	1.42 (0.57-3.58)	0.4535	0.41	3.60 (1.58-8.20)	**0.0023**
rs6064389	20	54,968,735	*AURKA*	0.40	1.35 (0.54-3.35)	0.5198	0.37	2.67 (1.27-5.64)	**0.0098**
rs6064391	20	54,971,386	*AURKA*	0.41	1.43 (0.64-	0.3779	0.38	2.56 (1.26-	**0.0094**

		3.19)	5.22)

Note: In the column titled “gene”, the numbers in parentheses indicate the distance from the SNP to the gene. All other SNPs are within the gene listed in that column. Adjusted Odds Ratios (aOR) and 95% Confidence Intervals (CI) are for the listed SNP compared to the wild type. The aORs were generated controlling for gestational age at delivery. Results are only listed for genes with $p<0.01$ for association.